Proteomic Investigations of the Ethylene Response in *Komagataeibacter* spp.

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Abstract

Komagataeibacter species are associated with fruits. Ethylene, a key phytohormone released from fruits during ripening triggers a positive feedback response causing the break down of starches into simple sugars. While a putative ethylene signaling pathway has been established in plants, little is known about the response pathway in *Komagataeibacter* despite its intimate relationship with fruit. Our lab has previously shown that *Komagataeibacter* responds to ethylene at the RNA level and has a direct effect on several genes involved in bacterial cellulose biosynthesis. The aim of this work was to investigate the proteomic response of *K. xylinus* and *K. hansenii* to ethylene and to identify key proteins in the bacterial ethylene response pathway. To do this, periplasmic and outer membrane extracts were prepared from cultures of *K. xylinus* and *K. hansenii* grown for 6 days in Schramm Hestrin (SH) medium, SH pH 6.8 and SH pH 6.8 supplemented with 1 mM ethephon, a compound which degrades into ethylene *in situ.* Differential protein expression was observed by electrophoresis of crude protein extracts on denaturing polyacrylamide gels. These results will be extended to further our knowledge of how plants and microorganisms coexist in nature.

Introduction

Ethylene governs plant growth, senescence and fruit ripening. In *Arabidopsis thaliana,* the putative ethylene signaling pathway involves five proteins (Hua *et al.* 1998) (Figure 1A). *Komagataeibacter xylinus* and *K. hansenii* are bacterial cellulose (BC) producing acetic acid bacteria (Yamada *et al*. 2012) which grow in close association with fruits. Recently, our lab has shown that these bacteria can produce ethylene and that exogenous ethylene causes differential gene expression within the BC synthesis operon (Augimeri and Strap 2015). Despite its importance to plant-microbe interactions, very little is known about the bacterial ethylene response pathway (Figure 1B). In this study, fruit ripening by *Komagataeibacter* spp. and their proteomic profiles in response to exogenous ethylene were investigated. A deeper understanding of bacterial ethylene response will have significant agricultural applications.

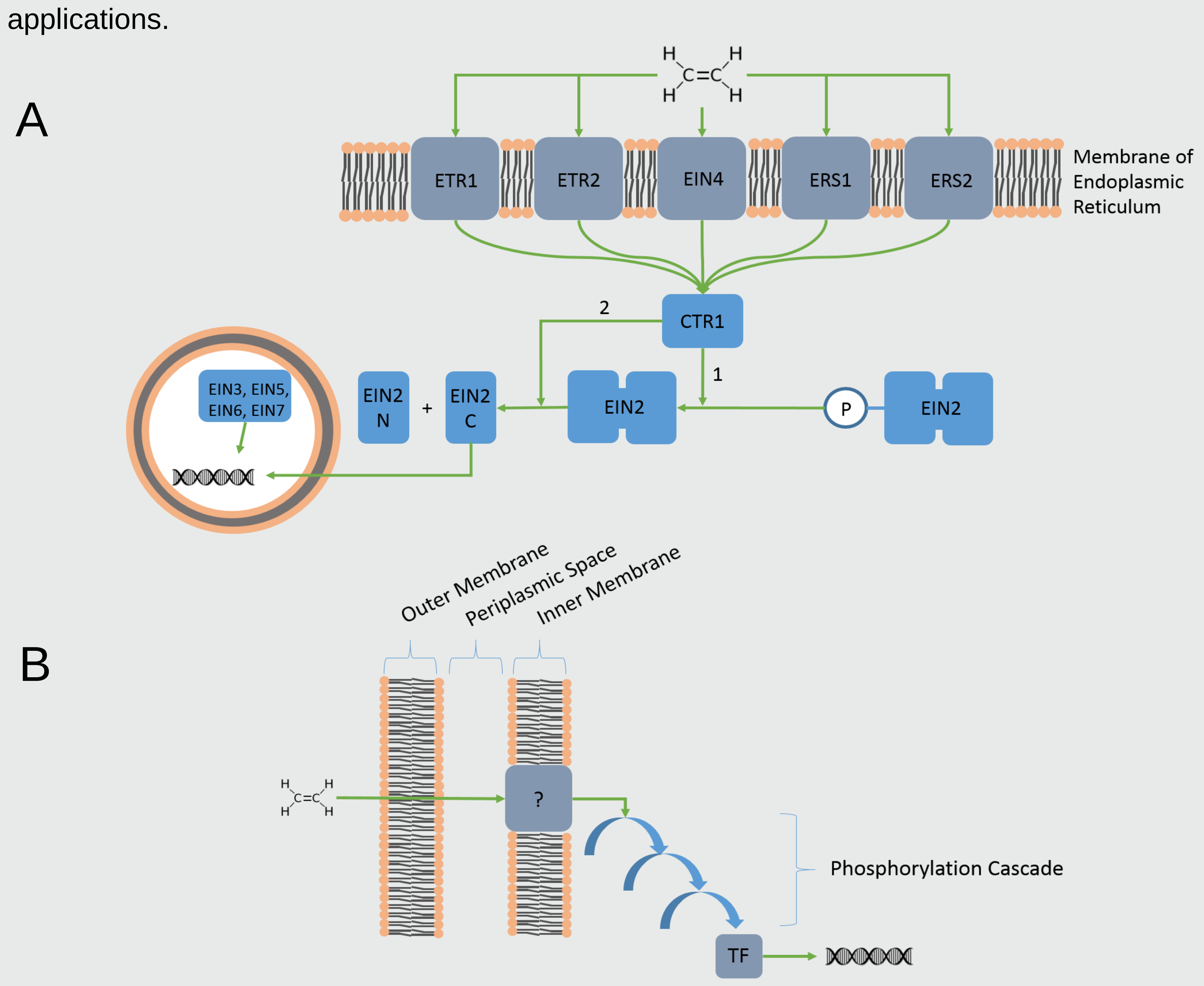

Figure 1. Ethylene response in plants and bacteria. A) Schematic representation of the plant ethylene signaling pathway. Ethylene can readily diffuse across membranes due to its charge and size (Chen *et al.* 2002). Ethylene diffuses into the cell and binds to receptor proteins on the endoplasmic reticulum which results in a phosphorylation cascade. In *A. thaliana,* the ethylene response phenotype is a thick, short hypocotyl and a more defined apical hook (Harvey *et al.* 1915). B) Schematic of the hypothesized bacterial ethylene signaling pathway.

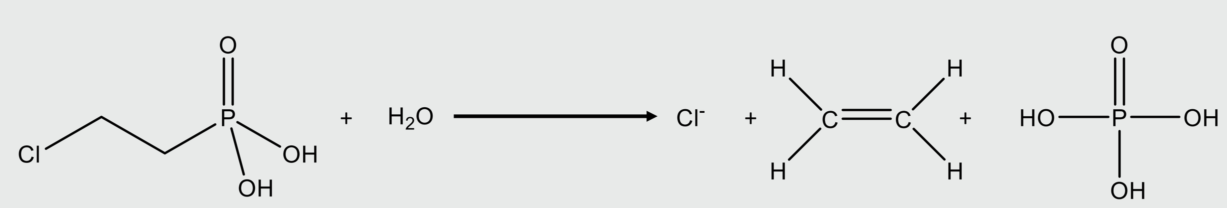

Figure 2. Decomposition of ethephon into ethylene gas. This reaction occurs above pH 3.5.

Methodology

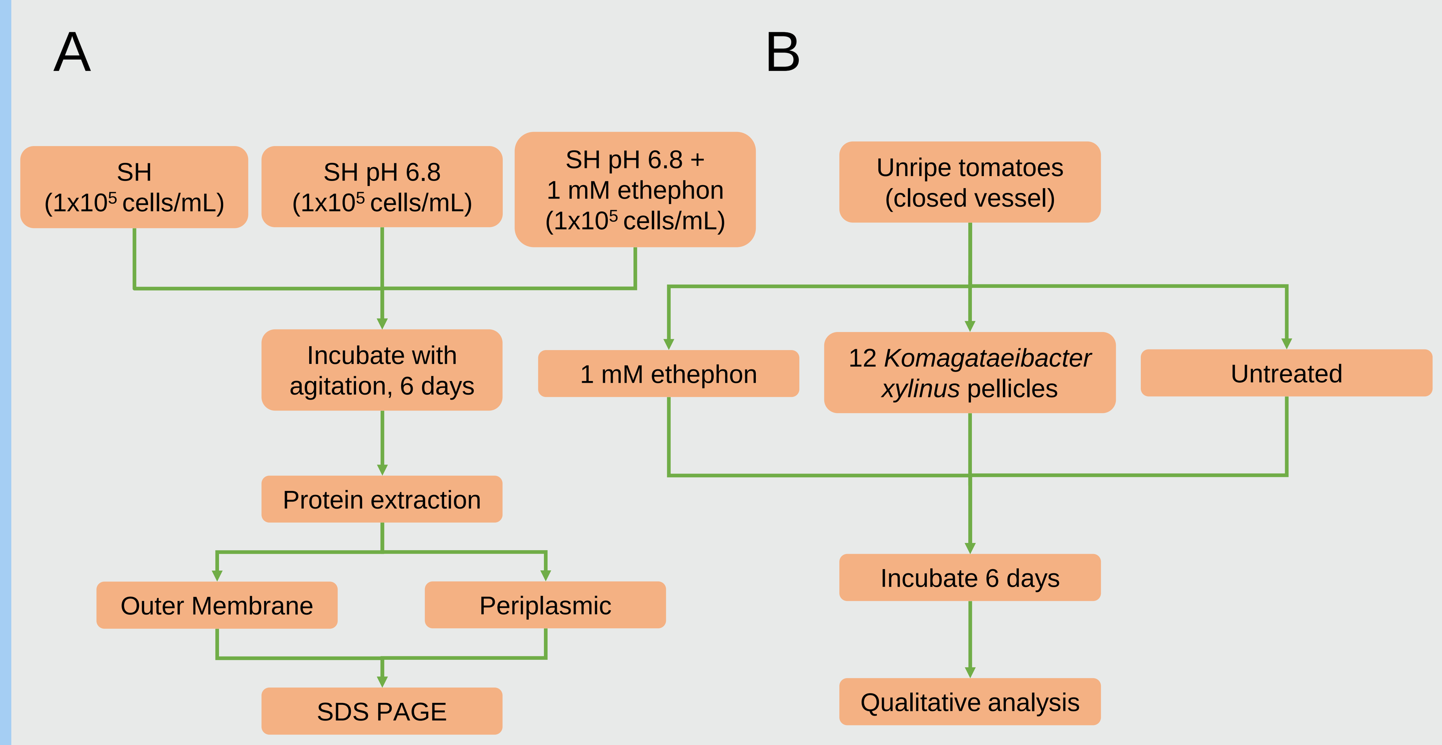

Figure 3. Methods used in this study. A) Scheme for the proteomic comparison of *Komagataeibacter hansenii and Komagataeibacter xylinus* grown in the presence and absence of ethephon-derived ethylene. B) Scheme for investigating the ripening of tomatoes by *Komagataeibacter xylinus* pellicles.

Results

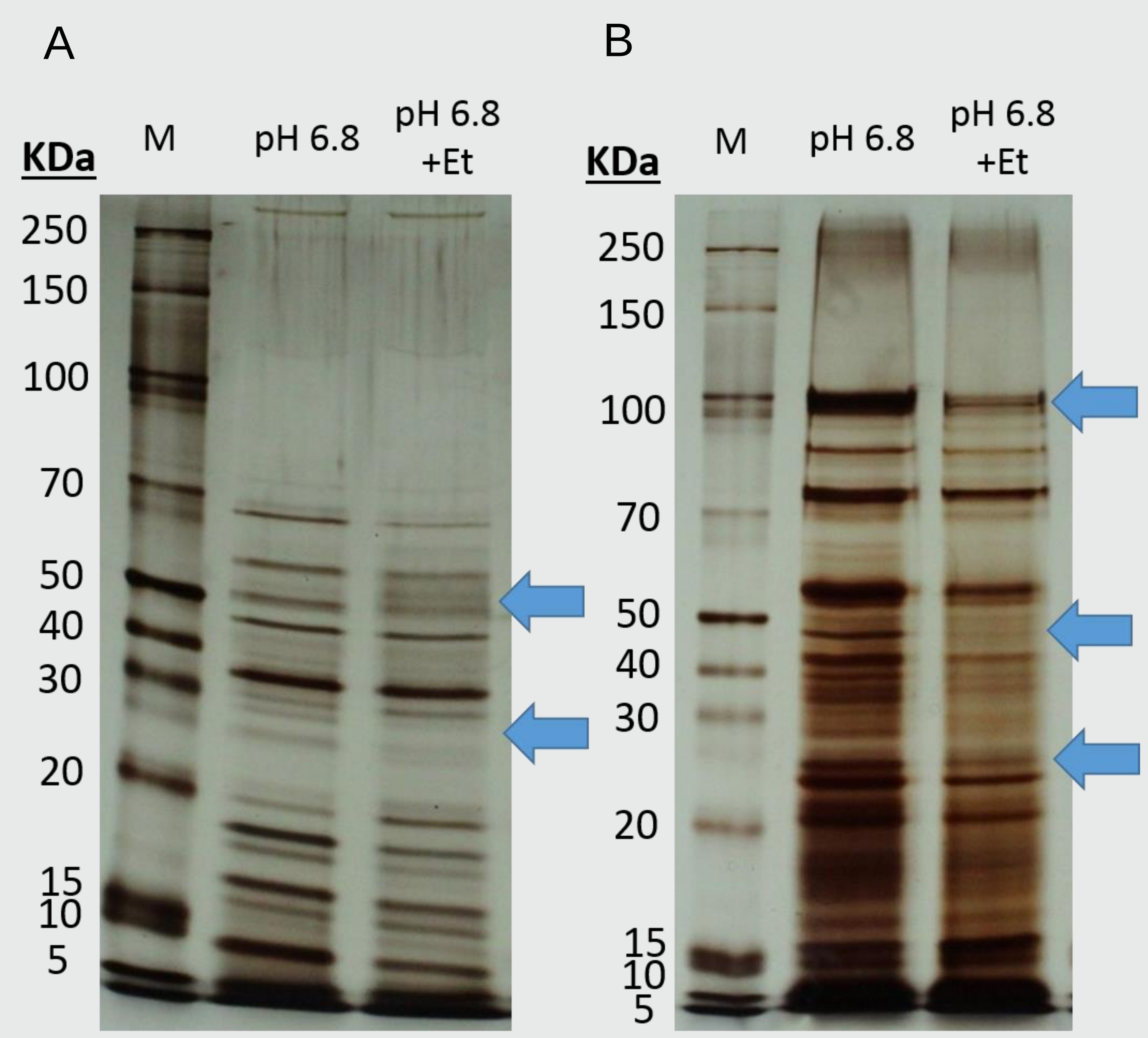

Figure 4. Periplasmic protein expression is affected by ethephon-derived ethylene and differs between species. A) SDS-PAGE comparing periplasmic protein expression in *Komagataeibacter hansenii* in the presence of ethephon-derived ethylene. Arrows denote notable protein expression differences. From left to right: molecular weight ladder, SH pH 6.8, and SH pH 6.8 supplemented with ethephon (Et). B) SDS-PAGE comparing periplasmic protein expression in *Komagataeibacter xylinus* in the presence of ethephon-derived ethylene. Arrows denote notable protein expression differences. From left to right: molecular weight ladder, SH pH 6.8, and SH pH 6.8 supplemented with ethephon (Et).

Results

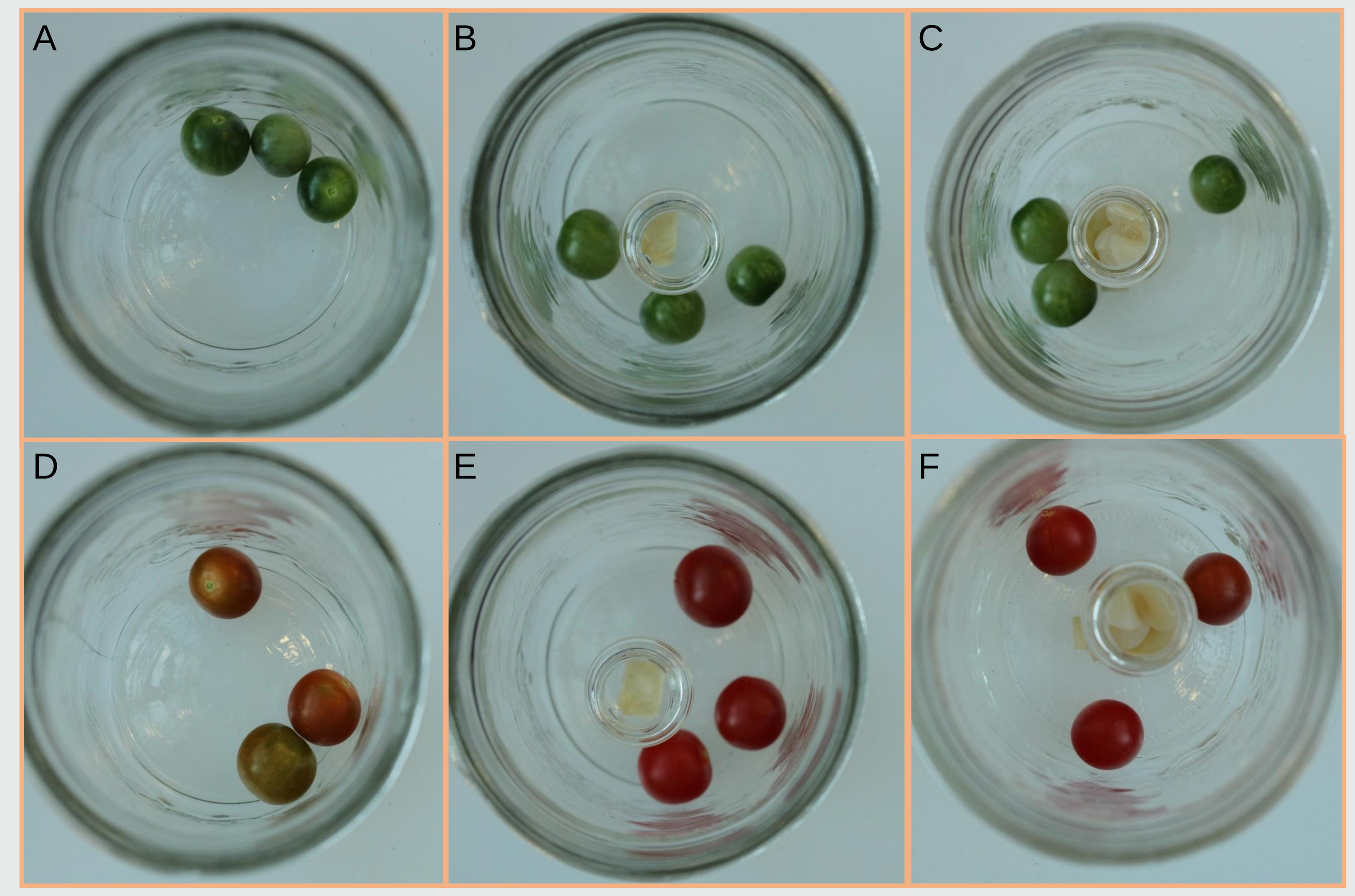

Figure 5. *Komagataeibacter xylinus* hastens tomato ripening. Unripe tomatoes at t=0, A) no treatment B) 1 mM ethephon, C) *Komagataeibacter xylinus* pellicles. Tomatoes after 6 days of incubation with D) no treatment, E) 1 mM ethephon, F) *Komagataeibacter xylinus* pellicles.

Discussion/Conclusion

1. Periplasmic proteins were found to be differentially expressed when *Komagataeibacter hansenii* and *Komagataeibacter xylinus* were grown in the presence of ethephon-derived ethylene.
2. Species differences were observed in response to ethephon-derived ethylene in periplasmic protein profiles.
3. Identity of differentially expressed proteins will be determined by protein sequencing.
4. *Komagataeibacter xylinus* can hasten the ripening of climacteric fruit.

References

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